

CLAIMS:

1. A novel microorganism *A. ureafaciens* K2032, which shows an activity of selectively producing difructose dianhydride IV from levan and an activity of degrading levan.

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2. A novel levan fructotransferase of the following amino acid sequence 1, derived from *A. ureafaciens* K2032, which can hydrolyze levan to selectively produce difructose dianhydride IV:

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1 M T P A I S R R A V L Q G A G A G A L A L I F G G A V P P A
31 A R A S A P G S L R A V Y H M T P P S G W L C D P Q R P V T
61 T H G A Y Q L Y Y L H S D Q N N G P G G W D H A S T T D G V
91 A F T H H G T V M P L R P D F P V W S G S A V V G T A N T A
121 G F G A G A V V A L A T Q P T D G V R K Y Q E Q Y L Y W S T

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151 D G G F T F T A L P D P V I V N T D G R A A T T P A E I E N
181 A E W F R D P K I H W D T A R G E W V C V I G R L R Y A A F
211 Y T S P N L R D W T L R R N F D Y P N H A L G G I E C P D L
241 F E I T A D D G T R H W V L A A S M D A Y G I G L P M T Y A
271 Y W T G T W D G E Q F H A D D L T P Q W L D W G W D W Y A A

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301 V T W P S I D A P E T K R L A I A W M N N W K Y A A R D V P
331 T D A S D G Y N G Q N S I V R E L R L A R Q P G G W Y T L L
361 S T P V A A L T N Y V T A T T T L P D R T V D G S A V L P W
391 N G R A Y E I E L D I A W D T A T N V G I S V G R S P D G T
421 R H T N I G K Y G A D L Y V D R G P S D L A G Y S L A P Y S

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451 R A A A P I D P G A R S V H L R I L V D T Q S V E V F V N A
481 G H T V L S Q Q V H F A E G D T G I S L Y T D G G P A H F T
511 G I V V R E I G Q A I *

3. A novel levan fructotransferase gene, which has a base sequence
30 coding for the amino acid sequence of claim 2.

4. A novel levan fructotransferase gene as set forth in claim 3, wherein the base sequence is the following sequence 2:

1 GCGGTGCACCCGACTTCCCTCGACGACCACTGTCCTACCGCCGACCGCCCGCGGCTGCTCCTCAGCCTAGACGGGCCCTC
5 91 CTCGAGGTCTTCGTGGGGACGGTGAGGCGACTGCGTCGAACCTGGTCTCTCTGGGGCCGGCGGTGTGACCGCGAGCCTCGAGACGGCA
181 CGGCCAGGAACCGTGACGTGACCGGATCGACGTGAGGCGCCAGCGATGCTGACGCCCTGAACCTGCCGCCGTTCTGGGCTGACGA
271 GCGCTCCACCCGACAGCTCTCTTCTACCGCTGCCGAACAGGGTGGACGCTTCGTGCGGCCACCGTCCACGAGAGGAACGACGA
361 ATGACGCCGGCCATCTACGCCGCGCGTCTCCAGGGAGCGCGCGGAGCACTCGCCCTGATCTTCGGCGGTGCTGTGCCGCTGCA
451 GCCCGGGATCCGCTCGGGCTCGCTCCGTGCCGTCTACACATGACGCCCCAGCGGCTGGCTCTGCGACCCCAACGCCCGGTACCC
10 541 ACCCAGCGCGCTACAGCTGTACTACCTGCACTCCGACCAGAAACAGGCCCGGGCGGTGGGACCACGCGAGCAGACCGACGGCGTC
631 GCCTTCACGACACCGGACCGTGATGCCGCTCGGCGCCGACTTCCCGTGTGGTCCGGGTGGCGGTGCTGGCACCCGGAACACGGCA
721 GGGTTCGGCGCGCGCGGTGCTCGCGCTCGGACCCAGCGACCGACGGCGTCCGCAAGTACCAGGAGCAGTACCTCTACTGGTCGACC
811 GACGGCGGTTCACTTCACCGCCCTGCCGACCCGTCATCGTCAACACCGACGGTCCGCGCCGACCAACGCCCGCGAGATCGAGAAC
901 GCCGAGTGGTCCGCAACCAAGATCCACTGGGACACCGCCCGGAGAATGGGTCTGCGTCATCGGACGACTGCGGTACGCCGCGTTC
15 991 TACACCTCGCCGAACCTGCGCGACTGGACACTTCGCGCAACTTCGACTACCCGAACACGCCCTCGGCGGCATCGAGTGCCCCGACCTG
1081 TTCGAGATCACCGACGACGCGGACACGCCACTGGGTGCTCGCCGCCAGCATGGAACGCTACGGCATCGGCCTCCCATGACGTACGCC
1171 TACTGGACAGCACCTGGGACGGCGAGCAGTTCCACGCCGACGACCTACCCCGCAATGGCTCGACTGGGCTGGGACTGGTACGGCGGC
1261 GTCACCTGGCATCGATCGACGCGCCGAGACCAAGCGCTCGCCATCGGTGGATGAACAACGGAAGTACGCCGACCGGACGTCCCC
1351 ACCGACGCATCGACGGCTACAACGGGCAGAACTCGATCGTCCGCGAGCTGCGGCTCGCCGACAGCCTGGCGGTGGTACACCTCCTG
20 1441 AGCACCCCGTGGCAGCGCTGACGAACCTACGTACCGCCACCACTACCTCCCGACCGGACCGTTCGACGGCAGCGCGTCTGCCATGG
1531 AACGGACGCGCATACGAGATCGAGCTCGACATCGCCTGGGACACCGCGACGAACGTGCGCATCTCGGTGGCGCGTCCCCGACGGAACC
1621 CGGCACACGAACATCGGCAAGTACGGAGCAGACCTGTACGTGACCGGAGGACCTCCGACCTCGCGGGTACTCGCTCGCCCCCTACTCG
1711 CGAGCCGCCGCCCATCGACCCCGCGCGCGATCCGTGCACTGCGCATCTCTGTCGACACCCAGAGCTCGAGGTCTTCGTCAACGCC
1801 GGCCACACCGTGTCTCCAGCAGGTCCACTTCGCCGAGGGCGACACGGGAATCTCGCTCTACACCGACGGCGGCCCGCACACTTCACC
25 1891 GGCATCGTCTCGCGAGATTGGCAGGCGATCTAGGCGATGCACACCAACCGCTCACCGAAGCCGCGCCCCGGGAGACGACGGCCGAC
1981 AATCGACACGTCTCTCGTCTT

5. A novel levan fructotransferase gene as set forth in claim 3, wherein the base sequence is the following sequence 3:

30 1 GCGGTGCACCCGACTTCCCTCGACGACCACTGTCCTACCGCCGACCGCCCGCGGCTGCTCCTCAGCCTAGACGGGCCCTC
AvaI AvaI
91 CTCGAGGTCTTCGTGGGGACGGTGAGGCGACTGCGTCGAACCTGGTCTCTCTGGGGCCGGCGGTGTGACCGCGAGCCTCGAGACGGCA
181 CGGCCAGGAACCGTGACGTGACCGGATCGACGTGAGGCGCCAGCGATGCTGACGCCCTGAACCTGCCGCCGTTCTGGGCTGACGA

271 GCGCTCCACCCCGACAGCTCTCCTTCTACCGCTGCCCGAACAGGGTGGACGCTTCGTGCGCCACCCGTCACGAGAGGAACCAGCA
PsiI
361 ATGACGCCGGCCATCTCACGCCGCCGTGCTCCAGGGAGCCGGCGCCGAGCACTCGCCCTGATCTTCGGCGGTGCTGTGCCGCTGCA
I M T P A I S R R A V L Q G A G A G A L A L I F G G A V P P A
5 451 GCCCGGGCATCCGCTCCGGGCTCGCTCCGTGCCGTCTACCATGACGCCCCAGCGGCTGGCTCTGCGACCCCCAACGCCCGGTCAAC
31 A R A S A P G S L R A V Y H M T P P S G W L C D P Q R P V T
541 ACCCAGCGCGCTACAGCTGTACTACCTGCACTCCGACCAGAACACAGGCCCGCGGCTGGGACCACGCGAGCAGCAGCCGACGGCGTC
61 T H G A Y Q L Y Y L H S D Q N N G P G G W D H A S T T D G V
631 GCCTTCACGCACACGGCACCGTGATGCCGCTGCCGCCGACTTCCCCGTGTGTCCGGGTCGGCGGTCTGCGGACCCGCGAACACGGCA
10 91 A F T H H G T V M P L R P D F P V W S G S A V V G T A N T A
721 GGGTTCGGCGCCGCGCGGTCTGTCGCTCGCGACCCAGCCGACCGCGTCCGCAAGTACCAGGAGCAGTACCTCTACTGGTCGACC
121 G F G A G A V V A L A T Q P T D G V R K Y Q E Q Y L Y W S T
811 GACGGCGGGTTACGTTACCGCCCTGCCGACCCCGTCATCGTCAACACCGACGGTCGCGCCGCCACCACGCCCGCCGAGATCGAGAAC
151 D G G F T F T A L P D P V I V N T D G R A A T T P A E I E N
15 901 GCCGAGTGGTTCCGCGACCCCAAGATCCACTGGGACACCGCCCGGAGAATGGGTCTGCGTCATCGGACGACTGCGGTACGCCGCGTTC
181 A E W F R D P K I H W D T A R G E W V C V I G R L R Y A A F
991 TACACCTCGCCGAACCTGCGCGACTGGACACTTCGCCGCAACTTCGACTACCCGAACACGCCCTCGGCGGCATCGAGTGCCCGACCTG
211 Y T S P N L R D W T L R R N F D Y P N H A L G G I E C P D L
1081 TTCGAGATCACGCGAGACGCGGACACGCCACTGGGTGCTCGCCGCCAGCATGGACGCCTACGGCATCGGCCTCCCCATGACGTACGCC
20 241 F E I T A D D G T R H W V L A A S M D A Y G I G L P M T Y A
1171 TACTGGACAGGCACCTGGGACGGCGAGCAGTTCACGCGGACGACCTCACCCCGCAATGGCTCGACTGGGCTGGGACTGGTACGCGGCC
271 Y W T G T W D G E Q F H A D D L T P Q W L D W G W D W Y A A
ClaI Aval
1261 GTCACCTGGCCATCGATCGACGCGCCGAGACCAAGCGCCTCGCCATCGCGTGGATGAACAACTGGAAGTACGCCGACGCGACGTCCCC
25 301 V T W P S I D A P E T K R L A I A W M N N W K Y A A R D V P
1351 ACCGACGCATCCGACGGCTACAACGGGCAGAACTCGATCGTCCGCGAGCTGCGGCTCGCCCGACAGCCTGGCGGCTGGTACACCTCCTG
331 T D A S D G Y N G Q N S I V R E L R L A R Q P G G W Y T L L
NcoI
1441 AGCACCCCGTGGCAGCGCTGACGAACTACGTACCGCCACCACCACTCCCCGACCGGACCGTCGACGGCAGCGCGCTCCTGCCATGG
30 361 S T P V A A L T N Y V T A T T T L P D R T V D G S A V L P W
1531 AACGGACGCGCATACGAGATCGAGCTCGACATCGCCTGGGACACCGCAGCAACGTCGGCATCTCGGTGGCGCGCTCCCCGACGGAACC
391 N G R A Y E I E L D I A W D T A T N V G I S V G R S P D G T
1621 CGGCACACGAACATCGGCAAGTACGGAGCAGACCTGTACGTGACCGAGGACCCTCCGACCTCGCCGGGTACTCGCTCGCCCCCTACTCG
421 R H T N I G K Y G A D L Y V D R G P S D L A G Y S L A P Y S
35 1711 CGAGCCGCCGCCCATCGACCCCGCGCGCGGATCCGTGCACCTGCGCATCCTCGTCGACACCCAGAGCGTCGAGGTCTTCGTCAACGCC

451 R A A A P I D P G A R S V H L R I L V D T Q S V E V F V N A
 1801 GGCCACACCGTGCTCTCCAGCAGGTCCACTTCGCCGAGGGCGACACGGGAATCTCGCTCTACACCGACGGCGGCCCGCACACTTCACC
 481 G H T V L S Q Q V H F A E G D T G I S L Y T D G G P A H F T
 SmaI
 5 1891 GGCATCGTCGTCGCGAGATTGGCCAGGCGATCTAGGCGATGCACACCACACCGCTCACCGAAGCCGCGCCCCGGGAGACGACGGCCGAC
 511 G I V V R E I G Q A I *
 1981 AATCGACACGTCCTCGTCGTT

6. A recombinant expression vector, carrying the levan fructotransferase
 10 gene of claim 3.

7. A recombinant expression vector as set forth in claim 6, wherein said
 expression vector is pUDFA81.

8. A novel transformant transformed with a recombinant expression
 15 vector as set forth in claim 6.

9. A novel organism *E. coli* JUD81 (KCTC 0877BP), which is prepared
 by transforming *E. coli* DH5 α with the expression vector pUDFA81 of claim 7.

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10. A process for producing difructose dianhydride IV from levan, in
 which a levan solution is subjected to reaction at 25-50°C for 3-10 hours in an
 acidic buffer of pH 3.0-7.0 in the presence of a levan fructotransferase.

11. The process as set forth in claim 10, wherein the reaction is carried
 25 out at 37°C.

12. The process as set forth in claim 10, wherein the acidic buffer is a

phosphate buffer of pH 5.8

13. The process as set forth in claim 10, wherein the levan solution has a levan concentration of 5-15 % (w/v).

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14. The process as set forth in claim 10, wherein the levan fructotransferase has histidine residues at its C- or N-terminus.

15. A process of preparing levan fructotransferase, comprising the steps
10 of:

culturing a bacterial species anchoring a levan fructotransferase gene-carrying, expression plasmid;

harvesting and homogenizing the cells; and

isolating levan fructotransferase from the cell homogenate.

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16. The process as set forth in claim 15, wherein the levan fructotransferase has histidine residues at its N- or C-terminus.

17. The process as set forth in claim 15, wherein the isolating step is
20 carried out using metal ion-affinity chromatography.

18. A process for producing difructose dianhydride IV from sucrose, comprising the steps of:

25 reacting a sugar solution at room temperature or lower in an acidic buffer of pH 3.0-7.0 in the presence of a levansucrase to produce levan;

purifying the levan from the sugar reaction mixture, partially or completely;

reacting a levan solution at 25-50°C for 3-10 hours in an acidic buffer of pH 3.0-7.0 in the presence of a levan fructotransferase to produce difructose dianhydride IV; and

isolating the difructose dianhydride IV from the levan reaction mixture.

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19. The process as set forth in claim 18, wherein the levansucrase is derived from *Z. mobilis*.

20. The process as set forth in claim 18, wherein the sugar solution is
10 reacted at 0-15°C.

21. The process as set forth in claim 18, wherein the sugar solution has a sugar concentration of 10-30 % (w/v).

15 22. The process as set forth in claim 18, wherein the levan solution is reacted at 37°C.

23. The process as set forth in claim 18, wherein the acidic buffer for the sugar solution is an acetic acid buffer of pH 5.0 and the acidic buffer for the
20 levan solution is a phosphate buffer of pH 5.8

24. The process as set forth in claim 18, wherein the levan solution has a levan concentration of 5-15 % (w/v).